NOTES ON THE PHYSIOLOGY OF REGENERATION OF PARTS IN PLANARIA MACULATA.[1]

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I. *Modes of Regeneration.*

In Planaria maculata there are two methods of inducing regeneration. First, isolated parts of sufficient size taken from any part of the body except the region in front of the eyes will regenerate; and second, partly isolated areas may regenerate, producing compound planarians.

1. *Isolated Parts.* — Randolph,[2] 1897, states that when a worm was cut into eight pieces by cross cuts, seven of them lived, and six of them regenerated all lost parts. The seventh failed to regenerate eyes.

Morgan[3] has shown that there is a limit of size below which regeneration of lost parts will not take place. He also thinks that while the area in front of the eyes, which does not regenerate, is near this lower limit of size, there is another cause, probably that of greater specialization, why it will not regenerate lost organs.

My own observations on regeneration of isolated parts, though limited, for the most part support those of Morgan, as will be seen from the following record of experiments. A worm 10 mm. long and 2 mm. to 3 mm. in width was cut into

[1] The work herein recorded was done in the Laboratory of Experimental Morphology of Michigan University, under the direction of Dr. F. R. Lillie, to whom the writer wishes to express his sincere thanks for assistance and encouragement.

[2] "Observations and Experiments on Regeneration in Planarians," Separat-Abdruck aus dem *Archiv für Entwickelungsmechanik der Organismen.* Bd. v, p. 355. 1897.

[3] "Experimental Studies of the Regeneration of Planaria maculata," Separat-Abdruck aus dem *Archiv für Entwickelungsmechanik der Organismen.* Bd. vii, pp. 365–372. 1898.

eight pieces as nearly equal in size as possible. All pieces regenerated lost parts and became fully developed worms in about ten days at ordinary room temperature. Another worm 5 mm. long and 1 mm. wide was cut into eight pieces. The operation was, however, so delicate that there was not much certainty in obtaining uniform size of the pieces. The larger pieces regenerated the lost organs, while the smaller ones did not. Just what the limit is, was hard to ascertain, as the relation of the piece to the whole could not be accurately determined, on account of its constantly varying shape. Parts which, by the most careful measurements, were shown to be about one-twelfth of the size of the original animal, regenerated and became fully formed planarians, while those of smaller size did not. Experiments on sixteen worms resulted in the same way. The area in front of the eyes did not regenerate in a single case.

2. *Production of Compound Planarians.* — This may be brought about in two ways: (1) Parts separated by cuts made along or near the middle line will generally complete themselves by regeneration without much growth. (2) Even extremely minute strips partly isolated may grow out like buds, and when of sufficient size, develop the characteristic organs of the species.

There is, of course, no line of demarcation between these two ways, which are united by a series of intermediates.

a. *By Regeneration.* — When a worm was split through the middle line of the anterior part of the body, sometimes the partly isolated left half regenerated a new right half and the partly isolated right half a new left half, thus producing a worm with two complete heads (Fig. 1). A similar operation may be performed on the posterior part of the body, resulting in two tails (Fig. 2). The time required for the regeneration of two heads is fifteen to twenty days, varying somewhat according to temperature. The regeneration of double tails occurred in five to ten days.

On Dec. 24, 1898, a large planarian was operated on by splitting the tail, as indicated in Fig. 13, except that the cut did not extend through the pharynx but only to the region

just posterior to it. On Jan. 3, 1899, two fully formed tails had developed. On January 5, the animal divided by fission about 3 mm. in front of the point of union of the two tails. The part possessing the two tails regenerated a new head, producing the animal seen in Fig. 3.

On January 11, the anterior part of the worm was again split posteriorly, this time dividing the pharynx. Five days later, on January 16, the posterior end of the worm again divided off, and subsequently regenerated a new head, as seen in Fig. 4. The third and most anterior part of the original worm was split posteriorly, but the double tails

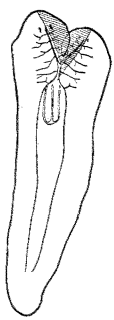

FIG. 1.

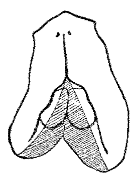

FIG. 2.

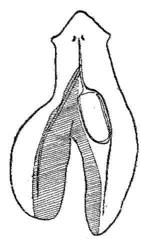

FIG. 3.

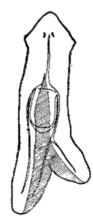

FIG. 4.

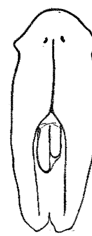

FIG. 5.

FIG. 1. — A double-headed planarian caused by regeneration after the original head had been split.
FIG. 2. — A planarian with two tails resulting from splitting and regeneration.
FIG. 3. — A planarian which separated from that of Fig. 5 by fission after its tail had been split.
FIG. 4. — This worm also separated from that of Fig. 5 by fission eleven days after Fig. 3.
FIG. 5. — The head end of the worm from which those in Figs. 3 and 4 separated.

could not be produced again, although the operation was performed three times. The only result that could be obtained was a worm with a slightly bifid tail and double pharynx (Fig. 5).

An interesting feature of the experiment with this worm is the way in which the alimentary canal developed in the regenerated parts. The left tail of Fig. 3 is supplied with nutriment by means of a sub-branch which comes off from the anterior branch of the canal, while the original left branch of the canal, which was severed in the operation, has disappeared. On the other hand, the right tail of Fig. 4 receives its nutriment by a sub-branch from the right posterior branch of the canal, which still persists, or is possibly a new formation. This and other problems concerning the anatomy of compound planarians are of interest and should be worked out.

b. *Budding.* — Small strips of tissue from the margin of the body or edge of a cut resemble buds in their capacity for growth and differentiation. These false buds regenerate more rapidly than larger portions of the body. To induce the growth of buds an incision is made, partly severing a very narrow strip (.5 mm.) of tissue, as shown by the lines in Figs. 6 and 10; Fig. 6 *a* indicates the method by which the worms in Figs. 8 and 9 were produced, and Fig. 10 *a* indicates how Figs. 11 and 12 originated.

In Fig. 7 the cut was made as indicated by the dotted line *a*. The bud, which was 4 mm. long, regenerated a new head, with brain, eyes, and cephalic lobes, in fourteen days. This head was developed from tissue in the posterior third of the body. Dalyell,[1] referred to by Randolph, thought that heads could be developed from tissue of the anterior part of the worm only. This idea is wholly disproved by Figs. 7, 11, and 12. At *b*, Fig. 7, is seen a bud one day after being cut.

i. *Growth.* — The bud, not having sufficient muscular strength to right itself against the larger part of the worm, heals without uniting with it, as is the case so often with animals split in the middle line. Growth begins very soon after the operation, being quite perceptible at the end of two days. It occurs in two ways: first, by regenerating new tissue on the cut edge of the bud; and second, by the increase of length, breadth, and thickness of the old tissue.

ii. *Differentiation of New Organs.* — Along with the increase in size the body becomes rounded off on the dorsal surface, and the head becomes broader and thicker in the region of the brain area when the cephalic lobes appear. Finally the eyes and pharynx, where a pharynx is developed, appear almost simultaneously.

In Fig. 8 the bud was formed by partly isolating a narrow strip of tissue from the side of the anterior part of the animal, as indicated by the shaded part *a*. About the time the cephalic lobes appeared, which was twelve days after the operation, the bud began to assert its independence, and was dragged about by the stronger worm with its head extending in a pos-

[1] "Observations and Experiments on Regeneration in Planarians," p. 370.

terior direction. As a result of the tension caused by the pulling, growth took place in the region *a* (Fig. 8), making the position of the posteriorly directed head a permanent and natural one.

In the case of Fig. 9 the bud was produced in the same way, but from day to day as the tension increased a slight cut was made at *a*, and as a result we do not have the head end of the bud directed posteriorly to the main axis of the worm, but nearly at right angles to it. The cutting prevented growth, and hence, when the animal comes to rest, or when relaxed in

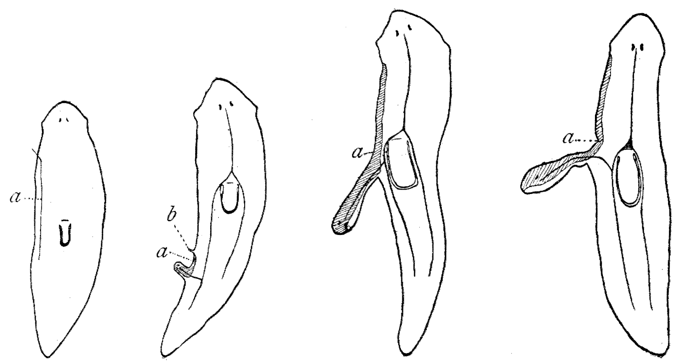

Fig. 6. Fig. 7. Fig. 8. Fig. 9.

Fig. 6. — At *a* is seen the kind of cut which was made to produce buds.
Fig. 7. — A head regenerated from a bud, and a bud, *b*.
Fig. 8. — Pseudoheteromorphosis.
Fig. 9. — Frequent cutting at *a* prevented pseudoheteromorphosis in the worm of this figure.

killing, the bud, instead of remaining in a posteriorly directed position as when in motion, takes a position more nearly the same as that which it originally occupied.

While the bud was developing, the cut edge of the larger part regenerated enough new tissue to replace that which went to produce the bud. Thus we have a well-formed double-headed planarian in the case of Fig. 8. In Fig. 9 the bud failed to develop a left eye. This may be due to the frequently cutting at *a*; otherwise our present knowledge of the case makes it impossible to decide what the cause may be.

iii. *Final Fate of Parts.* — One point was quite noticeable in all the experiments with buds. When the animal had be-

come well formed there was a strong tendency to divide and in this way get rid of the abnormal condition. Ignorance of this fact cost the writer two of his best examples of regeneration; and it was only by diligently watching their development and killing the material at the proper time that the examples for this part of the paper could be obtained.

II. *Heteromorphosis.*

1. *Historical.* — Randolph[1] mentions four cases found by Dalyell in 1811 which are worthy of mention here. The first was a planarian with a bifid tail, between the two branches of which was an erect structure supporting a head. Second, a planarian, upon the side of which incisions had been made, developed a head pointing downward in the direction of the tail. The third and fourth cases consist of two monstrosities, the description of which is quite similar to that of Fig. 11. These were two worms, each of which had another attached to it and lying at right angles to its tail.

Van Duyne[2] gives three figures which he claims prove the possibility of heteromorphosis in the planarian. One, his Fig. 3, represents a worm with two heads on the anterior part of the body, one of which points posteriorly. The second one, his Fig. 4, shows a worm whose body has been split through the tail almost to the head. Between the two tails thus produced two heads have appeared, which, when the tails are widely separated as represented in the figure, point in a posterior direction. And lastly, his Fig. 5 represents a tail pointing in an anterior direction.

Morgan[3] gives one example of apparent heteromorphosis, his Fig. 36. It shows a worm with two heads, which point

[1] "Observations and Experiments on Regeneration in Planarians," Separat-Abdruck aus dem *Archiv für Entwickelungsmechanik der Organismen*. Bd. v, p. 367.

[2] "Ueber Heteromorphosis bei Planarien," Separat-Abdruck aus dem *Archiv für die ges. Physiologie*. Bd. lxiv, Taf. x.

[3] "Experimental Studies of the Regeneration of Planaria maculata," Separat-Abdruck aus dem *Archiv für Entwickelungsmechanik der Organismen*. Bd. vii, p. 381.

in opposite directions when in a relaxed condition, but when expanded form an angle of about 100°.

Morgan [1] has confirmed Spallanzani's discovery of earthworms regenerating a tail in place of a head. Sections of these worms show a ventral cord extending to the new part, that no brain is present, and that the nephrostomes in the new part are turned backward towards the old part.

Loeb,[2] in his investigations to determine the cause of animal forms, produced monstrosities with hydroids in which the oral end was regenerated on the aboral end. Loeb proposed the term "heteromorphosis" for such monstrosities. Heteromorphosis not only includes the regeneration of a head in the place of a tail, but of any organ in any place where in nature one of unequal value would occur, as arms from the hips and legs from the shoulders, etc. Loeb defines heteromorphosis as "the replacement of one organ by another physiologically and morphologically different."

2. *Analysis.* — The term "heteromorphosis" thus includes the entire reversal of axial relations as well as the development of any single organ in place of another. It will be useful to distinguish these as polar heteromorphosis and heteromorphosis of single organs. Examples of the latter are found in various forms, as the regeneration of a tentacle-like organ in place of an eye in crabs, etc. Examples of polar heteromorphosis, on the other hand, with few exceptions, occur only among the Coelenterates.

Cerfontaine,[3] in his "Observations physiologiques sur l'Astroides calycularis," records the regeneration of a crown of tentacles on the base of a severed part of a polyp.

Loeb has found axial heteromorphosis to be quite common among the coelenterates and has been able to produce it in

[1] "A Confirmation of Spallanzani's Discovery of an Earthworm Regenerating a Tail in place of a Head," Abdruck aus dem *Anatomischen Anzeiger.* Bd. xv, pp. 407–410. 1899.

[2] *Untersuchungen zur physiologischen Morphologie der Thiere.* Bd. i, ii. Wurzburg, 1891.

[3] "Notes préliminaires sur l'organisation et le développement de différentes formes d'Anthrozoaides (deuxième communication)," *Bull. de l'Acad. Roy. des Sci., des Lettres et des Beaux-arts de Belgique.* No. 8, Notes v–viii. 1891.

at least the following forms: Tubularia mesembryanthemum, Aglaophemia pluma, Plumularia pinnata, Eudendrium (rasimosum ?), Sertularia (polyzonias ?).

Bickford and Driesch,[1] cited by Morgan, have also shown that in the tubularian hydroids two heads may develop on opposite ends of a piece cut from a stem, especially if the piece be short.

3. *Pseudoheteromorphosis.* — By cutting a narrow strip from any part of the body so as partly to isolate it, a posteriorly directed head may be developed by the reversal of the piece.

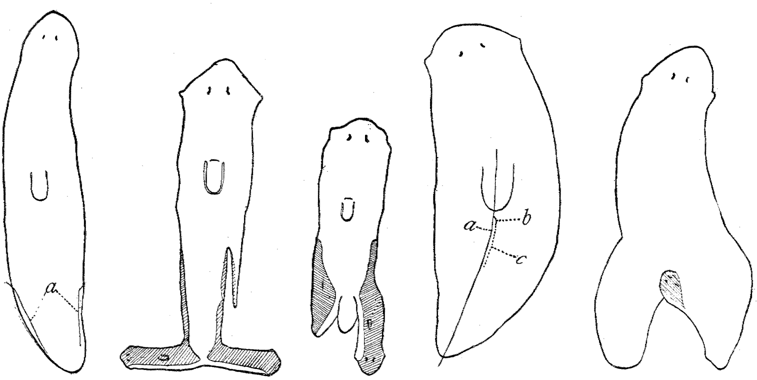

FIG. 10. FIG. 11. FIG. 12. FIG. 13. FIG. 14.

FIG. 10. — The lines *a* show the nature of the cuts which produced heads at right angles to the body.

FIG. 11. — A worm with heads lying at right angles to the main body.

FIG. 12. — Pseudoheteromorphosis.

FIG. 13. — The lines *a*, *b*, and *c* represent the cuts made to induce regeneration of heads in the tail region.

FIG. 14. — A head regenerated on one of the tails.

If by tension and growth this position becomes permanent, forms are produced which, to the casual observer, appear to be marked examples of heteromorphosis. Figs. 8, 12, 15, and 16 possess all the outward appearances of true heteromorphosis, but by the aid of Figs. 6, 10, and 13 one can readily show that there is neither the reversal of axial relations nor the development of one organ for another. Hence we do not have true heteromorphosis, but simply the swinging around of a portion

[1] "Experimental Studies of the Regeneration of Planaria maculata," Separat-Abdruck aus dem *Archiv für Entwickelungsmechanik der Organismen.* Bd. vii, p. 382.

of tissue as a whole, so as to give the anterior end a posterior direction, or pseudoheteromorphosis.

Perhaps the best example of pseudoheteromorphosis is found in Fig. 15, which was produced in the following manner. On Dec. 22, 1898, the worm was operated on by splitting its tail, as indicated by the line *a* in Fig. 13. Then a small anteriorly directed piece of tissue was partly isolated on the inner margin of the right tail *b*, which developed a head, as seen in Fig. 14, by Jan. 9, 1899.

Fig. 15 represents the same worm in an expanded condition during locomotion.

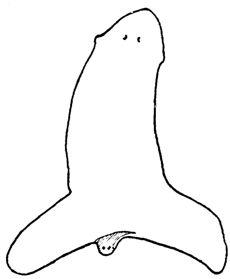

Fig. 15. — The worm of Fig. 14 in an expanded condition.

Fig. 16 was produced in the same way as Fig. 15, except that the strip, which was isolated from the inner edge of the right tail, was cut longer, as indicated by the dotted line *c* in Fig. 13.

Neither Van Duyne nor Morgan gives evidence of having produced anything other than pseudoheteromorphosis.

4. *Critique of Evidence.* — In Van Duyne's first example of heteromorphosis (Fig. 3 of the plate) he figures a worm with two heads, one of which arose from the wound caused by taking a piece from its side by two cuts, a transverse one back of the right half of the head, and a longitudinal one from the inner end of the first cut to the tip of the tail.

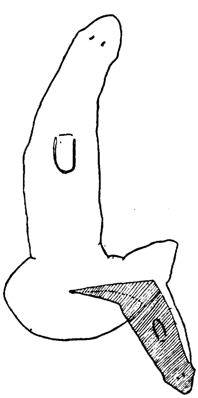

Fig. 16. — Pseudoheteromorphosis.

In order that this be an example of axial heteromorphosis it must have been regenerated from tissue which originally bore the same relation to the main axis of the worm as does the tail, *i.e.*, it must have been regenerated from tissue, the free end of which was originally posteriorly directed. The drawing does not clearly show this, but rather indicates that this head may have been regenerated on the anterior end of the newly formed tissue on the side of the worm and was forced to turn backward by the shoulder-like projection of old tissue.

Likewise in Fig. 4 of Van Duyne's plate there is no evidence that the heads may not have arisen from anteriorly directed tissue, as did the head in my Fig. 15. Fig. 5 of Van Duyne's plate gives no more evidence of being a tail than of being a partially developed head.

Morgan, in Fig. 36 of his paper, gives an example of what he considers to be axial heteromorphosis. He says: "The entire history of this piece is known, and there can be no doubt that two heads developed on opposite ends of the same cross-piece." Further he adds: "The bending of the heads to one side is due, in all probability, to the knife cutting somewhat obliquely to the long axis at the time that the piece was removed." May it not be more probable that we have here a case of the development of a head from each of the anterior corners of the piece? It is certainly reasonable to suppose this in the light of the evidence given. To determine whether this be an example of axial heteromorphosis or not, two things are necessary, *viz.*: (1) That we know the end of the piece which was originally directed anteriorly by some means other than the direction of its motion; and (2) that we know that the same end, which was the anterior end when the piece was first cut, continues to be the anterior end of the newly developed worm. Several cases were noticed where the piece, either from not having been cut squarely across or from some other cause, at first moved in a direction diagonal to its anterio-posterior axis, but afterwards, when the regenerated part developed normally, *i.e.*, in the line of the anterio-posterior axis, it again moved in a straight line. If the new tissue developed a little to one side of the anterio-posterior axis, as was sometimes the case, the piece continued to move in a diagonal direction, following the newly formed head. May not Morgan's Fig. 36 be an example involving conditions similar to these without involving axial heteromorphosis?

5. *Effect of Injury to One Part on a More or Less Different Part.* — In addition to the tendency to divide after the regeneration of new organs, referred to elsewhere, it sometimes happens that an operation on one part of the body produces an abnormality in some other part. Three interesting cases were

found where the eyes either divided, or became abnormally large and irregular in shape, and two where the pharynges developed abnormal proportions.

The first case was caused by an operation upon a planarian to produce a bud, as indicated in Fig. 6. Three times the bud divided off by fission, leaving the worm almost normal in appearance. After the third operation the eyes, which were crescentic in outline, began to deposit pigment in the concavity in irregular masses until the condition represented in Fig. 17 was produced, when the head separated from the body by fission.

The second case (Fig. 18) is that of a worm which had been operated on in a similar manner. The bud divided off and

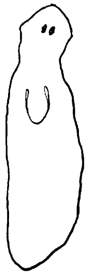

Fig. 17.

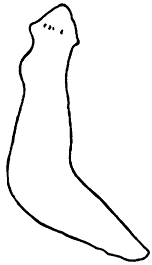

Fig. 18.

Fig. 19.

Fig. 17. — A worm in which the eyes have become abnormally large after being operated on.
Fig. 18. — A worm in which two new eyes developed after an operation upon its sides.
Fig. 19. — The dark part of the right eye divided after operation on the side of the worm.

almost immediately the eyes divided, giving four eyes. The one on the extreme left side of the head has the concavity on the right side, suggesting the possibility of its functioning as a right eye. Two others are in almost the normal position, while the fourth lies between them and a little to the left of the middle line of the head.

The third case (Fig. 19) is that of a worm which had been operated on in the posterior part of the body on the left side, producing a bud. When the bud had become half grown it divided near its anterior end. The right eye of the worm then divided in such a way as to produce two, one lying just anterior to the other. The head of this animal also separated from its body by fission soon after the division of the eye.

The two cases of abnormally developed pharynges were

found in two worms which had been split near the middle line of the body. One was split through the head back to the pharynx but not including it. After several operations two heads developed, and it was noticed that the pharynx was gradually increasing in width. This continued until the two heads were fully formed, when it had reached a size nearly twice that of the normal.

The other case was a worm whose tail had been split to the base of the pharynx. After the operation the pharynx increased continually in width until two tails were fully formed.

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